



## Predicting human minisatellite polymorphism.

France Denoeud, Gilles Vergnaud, Gary Benson

### ► To cite this version:

France Denoeud, Gilles Vergnaud, Gary Benson. Predicting human minisatellite polymorphism.. Genome Research, 2003, 13 (5), pp.856-67. hal-01158326

**HAL Id: hal-01158326**

**<https://hal-ensta-paris.archives-ouvertes.fr/hal-01158326>**

Submitted on 31 May 2015

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# Predicting Human Minisatellite Polymorphism

France Denoeud,<sup>1,4</sup> Gilles Vergnaud,<sup>1,2</sup> and Gary Benson<sup>3</sup>

<sup>1</sup>Laboratoire GPMS, Institut de Génétique et Microbiologie, Université Paris-Sud, 91405 Orsay cedex, France, <sup>2</sup>Centre d'Etudes du Bouchet, 91710 Vert le Petit, France, and <sup>3</sup>Department of Biomathematical Sciences, Mount Sinai School of Medicine, New York, New York 10029, USA

We seek to define sequence-based predictive criteria to identify polymorphic and hypermutable minisatellites in the human genome. Polymorphism of a representative pool of minisatellites, selected from human chromosomes 21 and 22, was experimentally measured by PCR typing in a population of unrelated individuals. Two predictive approaches were tested. One uses simple repeat characteristics (e.g., unit length, copy number, nucleotide bias) and a more complex measure, termed HistoryR, based on the presence of variant motifs in the tandem array. We find that HistoryR and percentage of GC are strongly correlated with polymorphism and, as predictive criteria, reduce by half the number of repeats to type while enriching the proportion with heterozygosity  $\geq 0.5$ , from a background level of 43% to 59%. The second approach uses length differences between minisatellites in the two releases of the human genome sequence (from the public consortium and Celera). As a predictor, this similarly enriches the number of polymorphic minisatellites, but fails to identify an unexpectedly large number of these. Finally, typing of the highly polymorphic minisatellites in large families identified one new hypermutable minisatellite, located in a predicted coding sequence. This may represent the first coding human hypermutable minisatellite.

[Supplemental material is available online at [www.genome.org](http://www.genome.org).]

Tandem repeats represent a significant fraction of vertebrate genomes and have been classified as satellites, minisatellites, and microsatellites according to the length of the repeated unit and the overall length of the array. Minisatellites are usually defined as the tandem repeats of a short (10- to 100-bp) motif spanning several hundred to several thousand base pairs and are associated with interesting features of genome biology (for review, see Vergnaud and Denoeud 2000).

Minisatellites frequently exhibit length polymorphism, which results from variation in the number of internal copies, making them valuable genomic markers. They provided the first highly polymorphic, multiallelic markers for linkage studies (Bell et al. 1982; Nakamura et al. 1987) and were used in the early stages of human genome mapping (NIH/CEPH Collaborative Mapping Group, 1992). Chromosomal distribution of minisatellites in the human genome is highly skewed toward telomeres and ancestrally telomeric regions (Amarger et al. 1998). Highly polymorphic minisatellites are thus a good tool for detection of microdeletions in the ends of chromosomes, associated with human pathologies such as mental retardation (Giraudeau et al. 2001). Polymorphic minisatellites are also found in bacterial genomes (Le Fleche et al. 2001), in which they have proven to be a powerful tool for bacterial strain identification.

Although the abundance of polymorphic minisatellites suggests that they are fast-evolving sequences, most of them are, in fact, quite stable. New alleles that display changes in the number of tandem copies have been observed at only a few loci, called hypermutable minisatellites. Changes at these loci in the germline can be observed in the next generation, and in humans, one locus, D2S90 (CEB1), has been found to

change in as many as 13% of the gametes (Vergnaud et al. 1991; Vergnaud and Denoeud, 2000). Hypermutable minisatellites may provide a potent source of information on the mechanism of minisatellite instability. In humans, this instability apparently arises at least in part through gene conversion events, during or shortly after meiosis, many of which involve interallelic transfers of information (Buard and Vergnaud 1994; Jeffreys et al. 1994; May et al. 1996; Buard et al. 1998). Similar intraallelic and interallelic recombination events are found in MS32 and CEB1 minisatellite sequences, when they are placed close to a meiotic hotspot in *Saccharomyces cerevisiae* (Appelgren et al. 1997, 1999; Debrauwère et al. 1999). Most likely, these events result from the gene conversion repair of double-strand breaks, as recent evidence indicates that meiotic recombination in mammals and yeast is initiated by the Spo11p endonuclease (Bergerat et al. 1997; Keeney et al. 1997; Baudat et al. 2000; Romanienko and Camerini-Otero 2000), which is also essential to the meiotic instability of the minisatellites introduced in yeast (Debrauwère et al. 1999). In agreement with these observations, it has been proposed that the meiotic hypermutability of some minisatellite structures is the byproduct of the coincidence of an ordinary minisatellite with a double-strand break hotspot (Vergnaud and Denoeud 2000).

Interestingly, hypermutable minisatellites might additionally provide biomarkers for low-dose exposure of the human germline to ionizing radiation (Dubrova et al. 1993, 1997; Dubrova and Plumb 2002). Unfortunately, <10 human hypermutable loci have been characterized so far, using approaches developed >10 years ago, whereas the population studies conducted to evaluate the effect of low-dose irradiation would greatly benefit from the availability of a larger panel of probes.

Given the multifaceted utility of minisatellites, determining which are polymorphic/hypermutable would seem a valuable task. Efficient tandem repeat detection software en-

<sup>4</sup>Corresponding author.

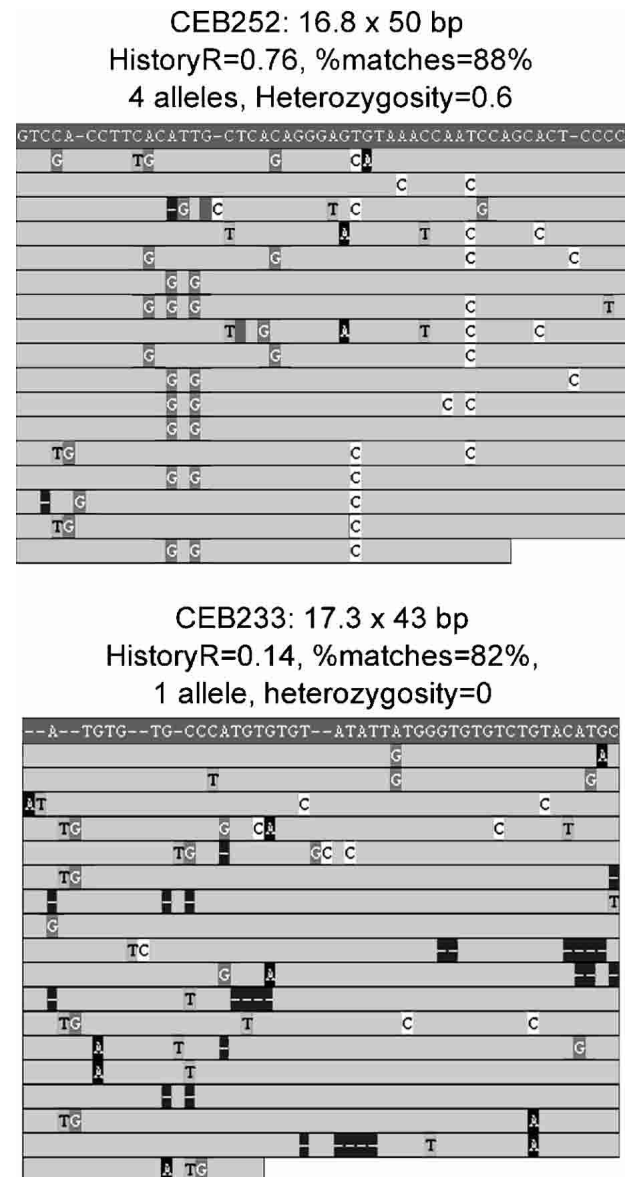
E-MAIL France.Denoeud@igmors.u-psud.fr; FAX 33-1-69-15-66-78.

Article and publication are at <http://www.genome.org/cgi/doi/10.1101/gr.574403>. Article published online before print in April 2003.

ables the identification of tandem repeats across entire genomes (Benson 1999; Vergnaud and Denoeud 2000), so that testing for polymorphism is all that is required. But although the polymorphism of the few dozen minisatellites usually present in a small genome can be systematically assayed at a reasonable cost (Le Fleche et al. 2001), this is not a realistic option for the human genome. There, the number of minisatellite loci is estimated in the thousands (based on the sequence of chromosome 22; Vergnaud and Denoeud 2000), the proportion of highly polymorphic minisatellites among these is not known, and previous efforts to identify hypermutable loci among minisatellites have produced only very low yields (~1% to 3% of those examined). Furthermore, sequence analysis of a few hypermutable loci has not yet revealed specific features that might facilitate their identification (Murray et al. 1999). Of need are predictive criteria that can be applied before the expensive and labor-intensive step of polymorphism typing.

Earlier attempts at polymorphism prediction for tandem repeats focused on microsatellites. Fondon III et al. (1998) identified polymorphic loci by selecting microsatellites in which the individual copies were at least 90% identical to a core pattern, but that study did not include a control group to test whether selection yielded higher polymorphism values than the background rate. Wren et al. (2000) improved polymorphic microsatellite identification by requiring perfect homogeneity of the repetitive unit. Such results are in accordance with the mutation process of microsatellites (replication slippage): They are stabilized by variant repeats (Weber 1990), the presence of which facilitates detection of slipped-strand DNA by the mismatch repair system (Strand et al. 1993; Heale and Petes 1995). In the case of minisatellites, in which internal conservation is not the rule at currently known hypermutable loci (Murray et al. 1999; Vergnaud and Denoeud 2000), such a high conservation requirement imposes too great a restriction on the set of potentially useful repeats and, as we report below, would preclude finding both highly polymorphic and hypermutable repeats.

The purpose of this report is to define inexpensive strategies to accelerate the search for highly polymorphic minisatellites. The goal has been the development of sequence-based predictive criteria for polymorphism. Results are based on the study of a representative pool of minisatellites selected from human chromosomes 21 and 22. Polymorphism for these loci was experimentally measured by typing in a population of unrelated individuals. This was followed by typing the most polymorphic loci across a number of large families to test for hypermutability. Two predictive approaches were tested. The most straightforward takes advantage of the availability of two different releases of the human genome sequence: one from the public genome sequencing project and the other from the private Celera project. The second approach uses sequence-based characteristics of the repeats—including such simple measures as unit length, copy number, degree of conservation, percentage of GC (%GC)—and a more complex measure based on the internal organization of variant motifs in the tandem array. A repeat that contains several distinct sets of nearly identical mutations exhibits *prima facie* evidence of multiple rounds of expansion and may be more likely to exist as multiple alleles than a repeat that contains mostly unique mutations (Fig. 1). This later measure is analyzed by using history reconstruction (Benson and Dong 1999), a type of parsimony analysis that infers how the present day sequence could have evolved from a single



**Figure 1** Multiple alignments of tandem repeats CEB252 and CEB233. In each alignment, the upper darker line is a consensus pattern for the basic unit, shown for reference, and the lighter lines are the individual copies, ordered from *top to bottom* as they occur in the repeat. Only differences with the consensus are shown. Heterozygosity for CEB252 is 0.6. Note several redundant patterns of mutation resulting in a high HistoryR score. Heterozygosity for CEB233 is zero. No clear organization of mutations resulting in a low HistoryR score.

ancestral copy while undergoing a minimum number of point mutations interspersed with duplications.

## RESULTS

### Characterization of Chromosome 21 and 22 Minisatellites

Human chromosomes 21 and 22 contain ~15,000 tandem repeats each (as detected by tandem repeats finder [TRF] in the

publicly available sequences that exclude heterochromatin; Benson 1999). For this study, the empirical definition of minisatellites follows the suggestion made in Vergnaud and Denoeud (2000), which is more stringent than the usual definition of minisatellites mentioned in the introduction: (1) unit length  $\geq 17$  bp, (2) copy number  $\geq 10$ , (3) total length  $\geq 350$  bp, (4) percent matches  $\geq 70\%$ , and (5) GC bias (i.e., strand asymmetry for G and C; see Methods section)  $\geq 0.35$ . This definition includes repeats clearly classified as minisatellites, not microsatellites, allows minisatellites shorter than the  $\geq 800$  bp usually identified by Southern blotting (Vergnaud 1989; Amarger et al. 1998) and removes repeats with highly diverged copies. On chromosomes 21 and 22, 127 tandem repeats fulfill these criteria. Table 1 indicates their position on the chromosomes. As described before for minisatellites derived by classical approaches (Amarger et al. 1998), they are mainly located toward chromosome ends (both chromosomes are acrocentric). Analysis shows no statistically significant differences between the minisatellites from chromosome 21 and 22 for any of the characteristics listed in Supplementary Table 1. The two chromosomes will subsequently be considered together.

### PCR Typing Results

Polymorphism results, that is, number of alleles observed and heterozygosity, are given in Table 1, as well as dbSNP accession numbers for the polymorphic minisatellites that were submitted to the SNP database (<http://www.ncbi.nlm.nih.gov/SNP/index.html>). Supplementary data about polymorphism is also available at <http://minisatellites.u-psud.fr>. For the minisatellites that were typed first ("training set"), the study was made on a population of 76 unrelated individuals. Results were comparable to those obtained with a subset of 28 unrelated individuals from the set of 76. Subsequent PCR typings (minisatellites from the "test set") were performed only on the 28 individuals, except for the most polymorphic loci that were typed in all 76 individuals in order to evaluate their polymorphism more accurately.

Among the 127 minisatellites, 118 were successfully amplified (55 on chromosome 21 and 63 on chromosome 22) by using the selected primer pair (Table 1). Not surprisingly, long minisatellites ( $>2$  kb) are the most difficult to amplify: Only five among eight were successfully amplified under the conditions used. Figure 2A shows the image of the gel obtained for minisatellite CEB285 on 32 individuals (including 28 unrelated individuals): Six different alleles can be assigned. About 75% of the minisatellites successfully amplified are polymorphic (i.e., two alleles or more), and 42% have a heterozygosity value  $\geq 0.5$ .

### Polymorphism Prediction: Sequence Characteristics and History Reconstruction

#### Training Set

Twenty-five out of 60 and 32 out of 67 minisatellites were picked randomly, from chromosomes 21 and 22 respectively, to be typed first: They form the training set. PCR amplification was successful on 51 out of 57. A comparison of the sequence and polymorphism characteristics between the training set and the remaining minisatellites showed that the two sets have comparable distributions except for percentage of matches, purine/pyrimidine bias, and GC bias. To determine if some sequence characteristics are associated with high

polymorphism, correlations between sequence characteristics and allele number or heterozygosity were calculated for the training set. The greatest correlations were obtained for HistoryR (a measure derived from the tandem repeats history reconstruction algorithm [Benson and Dong 1999]; see Methods section) and %GC (Fig. 3). Weaker correlations were also found for average entropy (strongly correlated with HistoryR), and unit length (data not shown). Based on these observations, we chose to test three predictive criteria: criterion 1, minisatellites with HistoryR  $\geq 0.54$ ; criterion 2, minisatellites with %GC  $\geq 48\%$ ; and criterion 3, minisatellites with HistoryR  $\geq 0.54$  and %GC  $\geq 48\%$ .

#### Test Set

Of the remaining 70 minisatellites, 67 were successfully amplified and used as a test set in order to confirm the predictive criteria deduced from the training set. For each of the three criteria, the test set was partitioned into two groups: a positive group fitting the predictive criterion and a negative group. Figure 4A illustrates the results: All three criteria are predictive, that is, heterozygosity and allele number are significantly higher in the positive group compared with the negative group. The best polymorphism prediction was obtained with criterion 3 (HistoryR and %GC combined). It produces an enrichment of repeats having heterozygosity  $\geq 0.5$  from 43% (29 of 67) in the test set to 59% (19 of 32) in the positive group and a diminishment of monomorphic repeats from 25% (17 of 67) in the test set to 6% (two of 32) in the positive group. Criterion 3 thus reduces by half (67 to 32) the number of minisatellites to type while eliminating most monomorphic minisatellites and keeping most polymorphic ones (Fig. 4A). One among five highly polymorphic minisatellites (heterozygosity  $\geq 0.85\%$ ) would have been missed using criterion 3.

### Polymorphism Prediction: Direct Sequence Comparison

The experimental polymorphism values measured here indicate that greatly enhanced efficiency of polymorphic loci identification is possible if the sequences of two independent alleles for each locus are available. The reasoning is that two random samples of a moderately or highly polymorphic locus will, with high probability, yield different alleles, whereas for a monomorphic or only slightly polymorphic locus, the alleles will likely be identical. Thus, selection based on observed allele difference in the two samples should enhance the proportion of loci obtained that are polymorphic. The applicability of this approach was directly tested by comparing sequences from the Human International Genome Sequencing Consortium (HGP) and Celera genomics. We establish selection criterion 4 to be different reported lengths in these two sequences. For the 127 minisatellites previously identified in the HGP sequence, repeat sizes in the sequence provided by Celera (Venter et al. 2001) were obtained by BLAST with the PCR primers. Three tandem repeats were not found in the Celera sequence, including two that were typed (CEB230, CEB256) and one long repeat (CEB215; length expected from HGP = 2834 bp) that could not be typed. Of the remainder, 51% (29 of 57) have a different length in the two sequences for chromosome 21 and 22% (15 of 67) for chromosome 22. From the measured heterozygosity values, we would expect 37% (43 of 116) to have different lengths between the two sequences, essentially the same as found. None of these



Table 1. List of the 118 Minisatellites That Were Typed: PCR Conditions, Polymorphism Results, and Allele Size Information

IDENTIFIER INFORMATION			PCR CONDITIONS			POLYMORPHISM			ALLELE SIZE INFORMATION		
Chr	Name	dbSNP ss#	Physical position (kb)	Left primer	Right primer	Annealing temperature (28ind)	Number of alleles (28ind)	Heterozygosity (28ind)	Length predicted by Human Genome Project sequence (bp)	Length predicted by Celera sequence (bp)	Observed size range (bp)
21	CEB256	6313628	220	CAACCTCCACTCCCGAGAAAAAG	CGTGCTGTGGAGCTATTACTTACTTGGAAA	68°C	3	0.57	1454	?	900-1450
21	CEB255		3948	CTGGAACCTCGACAAATTTTCAAGTGAGG	TTTTTGTGTGCGAACCTGACAAATTTA	55°C	1	0	1045	682	1050
21	CEB258		4445	ACATAACAATCAAGACAGAGCCTCACTGAC	TACATTTTCTGACTTCTGTGGTCTTCATGG	59°C	1	0	720	720	720
21	CEB260		7748	GATGTAGTTGATTGCTTGTAGTGCAATTAAC	ATCGCAAGTGTAAAGGTGTACTGTGGT	64°C	1	0	891	891	890
21	CEB261	6313629	10501	TGCAAAATCTCTCCCTCTCTGTGTTGATAAAA	GCTTATGTATGAGGCGACGAATAGATCAG	63°C	4	0.7	543	491	490-550
21	CEB263		19963	TTTTAAATTTCTGATTTTCTTTCGCAAGGTGA	CTTGCAGTATGAGGTCTCTGTATCTGGTC	68°C	1	0	917	917	920
21	CEB264		21556	GCACITTTGTCCCATGTGTCAATCTTAAC	AACACACAGAGAGCCGACGACAGAC	68°C	1	0	1086	1026	1090
21	CEB265	6313630	23006	CATACAGATACGGATGATTTCTTGTCTTTGG	TCTATCTTTTGAACCTCTGCCGTAGTCC	68°C	2	0.14	830	831	830-880
21	CEB234	6313631	23639	CGAGGTGCCCAAGGAGGGGAGG	GAGAGGCTGCCGTCCCGGATTGCT	68°C	2	0.36	636	636	610-640
21	CEB266	6313632	24912	GCACCAACCATAGAGCCACTGAGAT	ATTCTCTGGTATTTTTCATCTGGAAGCA	68°C	2	0.36	821	819	820-870
21	CEB268	6313633	26882	GGAAAGTGACAGCTCCCTGTTTGAATTAT	AAGACATAAGTGTCTCCAGGTGTTAACAGGA	68°C	6	0.64	498	357	500-690
21	CEB269		28940	CTGGAGGCCAGAAAGTTCAAAATCAAGTT	TATCCAAGTGGCTCTAGATCCAGTGATAC	68°C	14*	0.88*	1192	1044	1600-4500
21	CEB235		29314	GCITTCAGTGGCTCTGGAGGTTTTAGTAAG	AATAGCGAAGAGGATGTTCCCAACAAAAT	68°C	2	0.04	813	813	1500-1850
21	CEB270	6313634	30145	CTTGAGGAGGACTGAGCCCTTCAGAAGTTAG	TAAACTAGATGAAAGAGTGTCTGCGCCTGA	68°C	2	0.39	642	642	640-690
21	CEB271		30440	ACTCCTTGAGTCTTGGAGGACTGACAC	ACTACTCTCTGTTGGAGAGACACTCAC	57°C	10*	0.83*	2227	2038	1100-2900
21	CEB236		30516	TGCATTTTCTCTAGGGGAGTATGACAAAT	CTACTTGGATGCTGAGGCGAGGATTATG	68°C	1	0	899	904	900
21	CEB272	6313635	30670	AATTGTTGAGGAGACTTCATATGCTTTCC	GACATCCAAAAGGCCAATGAGTATATGAAA	59°C	2	0.47	600	498	600-640
21	CEB273		30771	CAGCTTGGCAATGGAGTGAGACTGTCT	ACTGCACTCCAGCTCTCCCATCCCTA	68°C	1	0	580	580	580
21	CEB274	6313636	30829	AAAGAGCCAAAGTGAAGTCCCTCTCTGTGAA	CTTCTGGGAACATCCATGGCTCAG	68°C	8	0.6	1241	1017	660-1420
21	CEB275		31147	AAACAAAGAGTCCAGGAGCGCTGAGAGA	AATCTGCTCTTGGCTCCATCTCTCAT	68°C	1	0	1508	1511	1510
21	CEB276	6313637	31252	GGTTTCTGGTCTGCAGTTTTTATCTGAGTT	AGTTTCAGTATTTTGAACAGCCAGATGGTA	68°C	2	0.19	838	541	840-870
21	CEB237	6313638	31420	ATGGAATCCAGAGAAGCAAGTTTACACCAAT	CAGTATCTCACCACTGCACCTCAGCAAGT	68°C	2	0.29	912	690	910-960
21	CEB238		31572	GAGTAGCCACAGGACAGAACTGAGAAAGC	CCTGAAGAAGAACAGAGGAGAAAGGATGAC	68°C	7*	0.79*	3109	7305	830-3100
21	CEB277		31619	TGTAATAATTCTATCCACCCAGATTGTATGC	ATCTTAAATGGCAGGTTAATGGATTGATG	57°C	1	0	1654	1657	1660
21	CEB239	6313639	31675	GAGACAGTATGACACACACAGACAAAAGC	CTGTACCCGGTTAGATCCACACCCCTATG	68°C	3	0.56	650	539	650-850
21	CEB278	6313640	31833	CTGAACGAAATTAAGTGTATACCCAGAAAGC	GGAATGTTTGAAGCAACCCGAATATACCAG	64°C	4	0.69	1033	1000	810-1200
21	CEB240	6313641	32177	AGGTGTACCAGTACAGCAGCTTTGGACCTTA	GTTTGTCTCTCTTGGCCCTCTGGAAAGTAA	68°C	3	0.51	1040	1040	900-1060
21	CEB279		32246	CTTCAGGAGGCTCTGTCTCTGGGTGAGAAG	AGAGTGTGTGTGCACAACCTGTCAAGTGA	68°C	1	0	761	763	760
21	CEB241	6313642	32529	AGAGCACACACCCACCTCAAAATCACTG	AGTGATGGACTGCAGATATTGGGACT	54°C	4	0.57	2953	1241	1980-2550
21	CEB242	6313643	32690	TTCAITTCCTGTGAAGCACAGCGTTT	AGAAAACAGGAGACTCACACGATCAACT	68°C	7	0.66	737	483	650-1200
21	CEB280	6313644	33109	CTGTGAACATTTTGTAGCCATGTGTGTGA	AAAGAGAAAAGAAAAGGAGGCTCATACC	68°C	2	0.07	589	590	590-670
21	CEB281	6313645	33286	GCTCAGTTCTCTCTCTATTCGACTTGGTC	ATGAAGCTGACGCGGGAAGATGGTTCT	68°C	3	0.37	635	635	600-670
21	CEB243	6313646	33317	CCCTAGGAGGGGAGGCTTAAGACCA	ATACCAGAGTCCAGCCCAAGTTAGCCGTTT	68°C	3	0.27	765	765	410-770
21	CEB244	6313647	33318	CTGCTGTAACCCAGGCTCACAAACCT	ACCCTAGATGACCCCTAGTGGGACCTACAC	68°C	2	0.17	643	644	640-860
21	CEB245	6313648	33383	TCTGTGGATAAACGTGAATATGCCCGAAAT	CCAGAATCCCAATCTCTGCCCAATG	68°C	2	0.04	901	901	850-900
21	CEB282	6313649	33481	AGAGGTATGAGCACAGGTGGTGAGAG	AGCATACACATCTCTGTTTGGGCATTA	57°C	3	0.23	645	647	610-710

(continued)



Table 1. (Continued)

21	CEB283	6313650	33711	AGAACTCTCTGTTCTCCCGCTGCT	GAAGAGAACTTTCAGATCAGACGAGGTT	68°C	4	0.71	1114	1045	1020-1110
21	CEB284	6313651	33832	ACTAAAGCAGTACTGGCTCCCTCCCTCT	AATCTAGTGCATTTCCGTAAGCGTGGT	68°C	3	0.55	1322	545	1300-1340
21	CEB246	6313652	33920	GATGCTGACTCAGTGGCTCTCTGT	ATCATTTAGATCCATGACTCCCTTGGG	68°C	5	0.66	758	758	600-1350
21	CEB247		34282	GTCACCTTGTGTTTCTGCCATCAG	CCCAGGGTTATAGACAATTTTGAACCTG	68°C	1	0	649	539	650
21	CEB285	6313653	34327	AACAGAGCTCCTGCTGATGAATAATTC	GTTTAGAGAGGAATGACGCGAGAGTGT	59°C	6	0.3	1092	1092	880-1090
21	CEB248	6313654	34384	CCTGTACATAGTGAAGGTGGTTCTATTG	GTAACCCACATCGAGAAAAAGGATGG	68°C	5	0.64	1510	1514	830-1800
21	CEB286	6313655	34390	ACTTCTCCACTCCTGGACATGCTAGTCT	CAAGACCCAGCTGTCTCCAGGAAT	64°C	5	0.66	713	713	560-850
21	CEB287	6313656	34454	ATCTAACTTTGAGGGCTTTGGTCT	AGCTGGAAGAAAGCAGCAGGGTCC	68°C	3	0.51	570	570	430-570
21	CEB288	6313657	34474	AATTAGAAGCAGTGAACACAGAGTGG	AAGACTAGTTCTTTGAGAGCAGCAAG	68°C	3	0.55	822	394	690-820
21	CEB249	6313658	34883	TTTTTGCCTTCGTAAGTAACAATTTCC	AAGCGAAGAGTGTGTGACAGTACTA	68°C	2	0.5	1305	430	1300-1350
21	CEB289		34741	ATTGCACTGTGTTATCTGATGTTGTTT	AATTACTTATTTCCGGCCCATCTGTGT	68°C	1	0	2144	3451	5000
21	CEB290	6313659	34830	GATACTTCTCCAGCAGGGGAAACAAGAA	CTGAGCAGGACAGAGGCTCTCATCT	68°C	4	0.64	833	374	670-920
21	CEB291		34854	ACAGCTCAAGTGGAGACAGGAAAC	GGAGCCCCCTCACAGGAGTAGATA	68°C	10	0.87	772	775	770-1300
21	CEB250	6313660	34932	CTTTGAGGTGAGTGGTGACTCTGCTC	CAGCAGCTAATTTCTAAGCTTAAAGTCTC	68°C	19	0.93	1045	487	540-1850
21	CEB292	6313661	34950	GGGACCTGCAATTTCCGTTTCAAGT	GAATCCCATGAGGAGCTGAGAGAG	60°C	5	0.55	647	648	580-1100
21	CEB251	6313662	34992	GGTGACAAAGTCCGACGTCAGTATTGAT	AATCATCTCTGGGAGGTGCCGTTTACATA	68°C	3	0.39	1737	?	1740-1960
21	CEB252	6313663	35084	TTTGGTGCAAGGTACAGATATCTCCTATG	GAATAATGTAATCAAGGGACAGGAAGAA	68°C	4	0.61	983	1033	980-1330
21	CEB253		35145	ACTCAGGCAGTTAGGGGTACACATCTCTAT	CAGACTTAAATTTCTCCCTTAAATTCACAA	62°C	6	0.49	798	663	870-1100
21	CEB254	6313664	35165	ATAACTAGGTTTCTTTGAGCAGCAGGAG	AAACTTAAAGAAACCGTGTAAATATGCCA	68°C	3	0.2	656	539	630-680
22	CEB224	6313665	1528	CTAGCCTACCCATCCCACTAGCTTACC	CAAGGAATCTGACTGGTAGTGGTGT	68°C	6	0.75	1066	1019	940-1500
22	CEB213		1571	CTACTTCCCTGCTTAGGAGCTAGCCATC	CAGTTATGAATCAATCAAGGCTTGTGCTG	68°C	5	0.53	1401	1092	900-1700
22	CEB293	6313666	3522	CCCATGTATGTGTGCTCTCTATCAT	CTGACACTCCATCAGTAGGAGTGGACACTG	64°C	3	0.35	858	858	820-910
22	CEB225	6313667	4095	TCTTCTCATTACAAAAGAGCAGTGTCAAA	AACTCCAGGAACTGGCAAGTCAGTCAG	59°C	2	0.23	555	555	550-600
22	CEB216	6313668	10395	GTTTCCCAATGCAAGTGTGTTGGTTATT	GGAGACTAACAGTGGCTACGGGATGTTTA	60°C	2	0.07	1563	1290	1300-1600
22	CEB294	6313669	11741	ACATTACCTCCATAGTGTGGCTGTGT	GAGGATGACCATGGGTATATGACAAA	68°C	3	0.5	624	628	630-800
22	CEB226		12766	AGCCAGAGGTTCAAGGCTACGATTAG	CACCCCGCTGTGTGTGTAACCTCT	68°C	1	0	650	652	650
22	CEB295		13055	ACCAATGTAATCACAGGCTCTTAGAGAGG	GAGAAATCCATTCTCTTGCTTTTCAACCT	68°C	9*	0.8*	805	809	1020-4700
22	CEB222		15802	AGCTTTTCTACACAGATACCCCTCACCTG	AAGGCCCCCAAGTCACTGGAATACAT	60°C	1	0	822	822	1200
22	CEB296	6313670	16228	GGACATGCTTGAGGGAATTTTACTTTGTC	AGAGGCTCTCCAGACAGCACTACAAT	68°C	5	0.52	1866	1868	1860-2200
22	CEB227		16333	CCCAAGGTACACAGGATGTTATATTTCTT	AAAGTGAGGACAGTAACACAAGGCTTATCG	68°C	1	0	670	671	670
22	CEB297	6313671	16658	ACCTGCCGTGATCTACATTCCTACCCAC	TCAGTAATGTTTGTCTCTCTCTCTCTCTCA	60°C	2	0.24	738	738	740-880
22	CEB298		18295	ATTAAGATACAGACAAAAGCAGGATGCTG	TAATCTTAGTCCACCCAGACATGCCCTAAG	68°C	8	0.78	569	567	1080-1300
22	CEB299	6313672	19972	CAACTCAGTCTCATTCCTCCACTCTGAGATT	TCCCAATTCCTACTTTAGAAAACCTTTGCGATT	68°C	8	0.79	1016	1993	1020-2500
22	CEB228		20986	GGGAACAACAATTATCACAGAGGCTTAATA	CTGAAGAGTGTGTGSCAAGGATGCTCT	68°C	1	0	723	723	700-720
22	CEB300		21044	AGATGGACAGAGAGCCAGGCTAAGT	GACACGCTCCAGGTGACCCCACT	68°C	3	0.56	968	758	1420-1670
22	CEB201	6313673	21327	ATCCCTGGTTCTGAAATCCTACGCTTC	AAGAGAGAAGGACCCAGACAATGTGGAC	68°C	3	0.58	820	2301	320-900
22	CEB301	6313674	21541	CTCAGGCTGCCCTACACGTTGAAATC	GTTGTCTGTTTGAAGGAAAAGGACTGTGT	60°C	3	0.33	1790	1796	1850-2090
22	CEB214		22157	GAGAGGTCAGCTATCAGGCCCATCC	GCTCCTGCCACCATGTCCATCTAAT	58°C	1	0	668	418	660
22	CEB302	6313675	22447	AAGTAAGGACTGAAAGGTGACGATCTTTGC	CTCTTACGAGTGGATGAGGCTCGTTTAT	68°C	2	0.04	737	739	680-740
22	CEB303	6313676	23529	GAAGCAAGAACACAGAGGATTTAGGATTC	CTTCTGCATCTCTGACCCCAAGCAT	60°C	2	0.13	713	713	710-770
22	CEB304		25144	GCCTGCCCTAGATGAAGTAGTTAGATC	ACAGGATCTCATGAACCTGAGTCACTGG	68°C	1	0	533	533	530
22	CEB229	6313677	26130	AGACCAATTAACACAGTGGGGTTAAAGG	TGTAAGGAGACATTAGCAAAACCAACGATT	68°C	2	0.5	500	500	500-520

(continued)

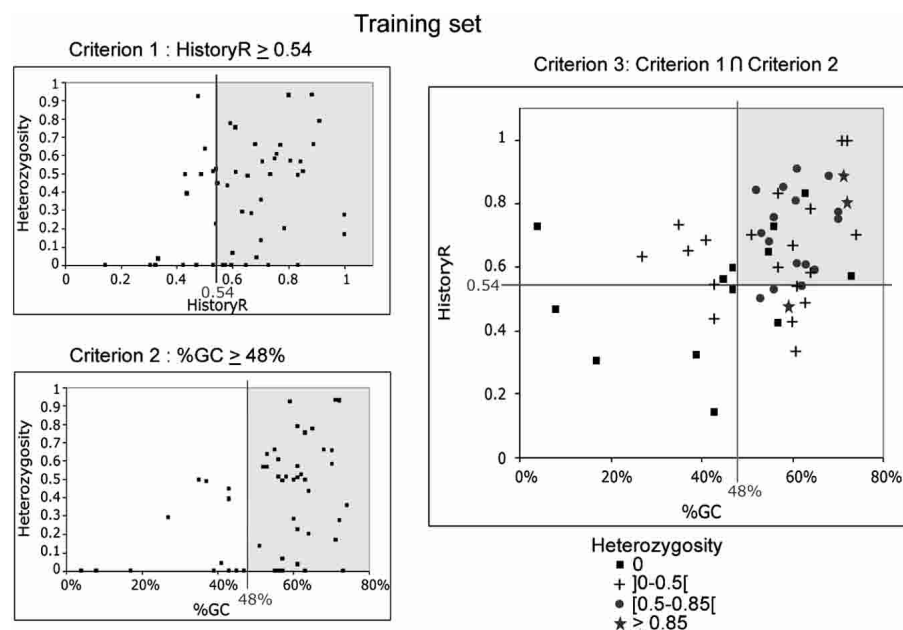


Table 1. (Continued)

22	CEB305	6313678	27086	CCACCGAACTTAAATATTCCACACATG	GTGAGCCATGAGGAGGAGGAGATGAG	68°C	14	0.89	1557	1109	1060-2150
22	CEB306	27209		CAGGTAGATGTTCCCAAGGTAGAACAGGT	CTAAACGGAAGGCCATTATCCAATGGTGAG	68°C	1	0	629	629	630
22	CEB307	6313679	27382	CAGCTTCAGAACTCAACCCCTGGTTCTAA	CTGAGCAGAGCAAGGACAATAATAGAGAC	68°C	4	0.58	528	528	430-640
22	CEB217	27398		CTGTGAGAAAGCATCTTCCTCTCTTGA	TACAGCTTCATCGCTGGTGGCTTTAGAC	68°C	1	0	1527	1527	1530
22	CEB308	6313680	27654	AGTAGCCTCAGTAAATCGAGAACTCTCCA	CTTAACCTAGCCCTTGCTCTCTGCTGAT	63°C	7*	0.78*	1191	1191	600-1200
22	CEB309	27773		CTCCAAACCAAAATCTCTATGACCCAAT	CAAAGTCACATGCTTTACCCCTCAACAAAG	58°C	1	0	513	513	513
22	CEB212	6313681	28490	CCCCAGCTGACCTACCTTGATCACTAT	TATAGTTGGTTTAGGCCCAACCATCTGTTA	68°C	4	0.49	1201	1203	1100-1400
22	CEB202	6313682	29067	AGAAAGGCTCAGCAAAATACAGTGTGAAC	ACTTTTCATCCCTCGCACAGCTCAG	60°C	19*	0.92*	907	909	630-1900
22	CEB230	30257		GTGTGTACGAGGCTGAGATCTAGGGATG	CGTGCCTCCACTGGTACTTTGACACC	68°C	5	0.77	883	884	720-1200
22	CEB231	6313683	30336	GAGTGTGCACTGAACCCATCTTTTATCAG	GTTCTGCTTCTGAGGGTAACTGGTTATG	68°C	3	0.45	1862	1844	1620-1850
22	CEB310	6313684	30541	CTTTTATGGCTAAGTAGTATTCOATCGT	CGTTCAGGGAAGAAAACAGAGATGACTT	68°C	12	0.85	998	998	900-1550
22	CEB311	6313685	30860	GTCTGGGTGGTTGCAAGTTGACAGTAG	CCAGCTGGATAAACTCTAAAGTCTCAGGA	68°C	2	0.46	1115	1111	1110-1180
22	CEB232	31534		CTAAACCATTTGTACCCCTCTGGAATTTGT	CATAAGTGTGAATTTGTTGGTGGTCTGAT	68°C	1	0	572	572	500-605
22	CEB312	6313686	31865	AGACTCTGCCAGGTGGAAATTTAAGATTGG	GCCTGATATCCAGAGAGAGATGCTTAG	68°C	2	0.04	615	615	610-640
22	CEB313	32217		ATGGTCAACAATAAACAACCCCATGTATT	GGCTGTTATCAGATTGTAGAGCAGAGCATC	64°C	1	0	811	811	810
22	CEB314	6313687	32267	AGAAGCTTGAAGACAAGACTGGAGTGCC	TCTGAGCTCTTCCCAGGTATTCACATATT	63°C	4	0.56	1037	1036	630-1040
22	CEB203	32298		AACCACTTCAATTTGAGAACTCGCTCTG	ACACCCCAACCCCATCTCTCTCTAT	68°C	1	0	695	695	700
22	CEB315	32458		AAAGACTCAGGGTTAAGGACAGAGAGAA	TAGGCCATCTTAAAGGAAGGACAGAG	68°C	5	0.72	1228	1245	1400-1600
22	CEB218	32693		ACCACACAGCCTCCGTGAATTTATTAGTA	AACCTACAACAGCACITGGAAACCCGAAG	68°C	1	0	590	590	600
22	CEB316	6313688	32741	ACAAAGATGGACCTGAATCACAATGAAT	AAAAATTTCCGCTGTTTAAAGCTGCCTGGAC	68°C	5	0.62	757	759	700-810
22	CEB219	6313689	32911	ACACTGAACATTTGGGAAGGGCTCTCTAC	CCTGTGGCTTCTCTCTCTCAGGTAAC	68°C	3	0.51	559	559	500-700
22	CEB317	32915		ACCTGAATATCGTTCAACCTCTGTATCT	AAAAAGTCTGGGAAAAAGCCCTCATCT	68°C	1	0	594	594	600
22	CEB204	6313690	32948	CAGTTCTCAAAACCCCAAGTGAAGTGA	CAGGATACTAGGGCTTAAGCATTTGGACA	68°C	5	0.66	875	867	850-1500
22	CEB318	6313691	32980	CACATACAGAAGAACAGCGGGAAGAACT	CACATGTGAGGCTGCATGGGAAGAAG	68°C	3	0.43	777	777	730-860
22	CEB205	6313692	33057	GGTTTTAGAAAGACAGGTGCAAGAAATTAGG	AGTGGTTAGGGTCTCTCTCTCTCAAT	68°C	19*	0.93*	1318	1317	550-2500
22	CEB206	6313693	33318	AGAACACAGCAGCTGAAATGCCATACC	CCTAACAAAGAAAGGTGAGAGTGTG	68°C	2	0.44	1227	1226	820-1270
22	CEB233	33400		TGCTAGGAATCCTTGTCTGTGTATATA	CAGCACATGGCAATTTGTTAGGATACACATA	68°C	1	0	856	856	850
22	CEB319	33414		ACTCTCCCTCCCATCTCCACTCTC	CTGCTGTTGCTTCTGCTCTCAGTTCTA	68°C	1	0	572	572	570
22	CEB320	6313694	33419	CTCCTCTCAGACCTGTCAAGACAACAAAC	GCTCATGAGAAACAGTAGATCCATCTGAG	68°C	2	0.04	691	691	690-760
22	CEB321	6313695	33434	GAAGAGACCTCATCATGGGCACTCC	ATCCTCTACTAGCCCTGATAGCACCACATC	63°C	5	0.62	1272	1272	1240-1500
22	CEB322	6313696	33545	CCTCACTGCTCATTTGCGATCAGACAT	GGTACTGCTCTTTGAGGACAGGGCTAACT	68°C	2	0.04	578	578	540-580
22	CEB220	6313697	33592	AAGACCAAGATCTGAACCTCAACTTCCT	GTGACTTGGCTTTTCCATCTCTCTCTGT	68°C	3	0.28	785	785	700-930
22	CEB223	33618		ATGGAGAGTGGGGCCCTTGATGTTAG	CTTTTCTGCAACCTTAAAGGGCATGTG	68°C	1	0	872	872	870
22	CEB207	6313698	33817	ACAACAATAAACAATCTGCCCCAACTC	AGGATTTCTTAACCTGTGACAGGGGATGCT	68°C	2	0.13	2617	2663	2600-3500
22	CEB324	6313699	33825	GTGGGGCAAGAGGCATCTCCGTGAGT	CGCCCGGCAATAGGGGGGGTCTTTA	68°C	19*	0.93*	985	244	400-3500
22	CEB325	6313700	33854	GGCCCTTCTCTCTGTTCCATACTG	CTCTGCTTCAGAAACCCCATACCTCT	58°C	4	0.53	926	928	930-1400
22	CEB221	6313701	33864	CAAAATAATTGGAGTAGGAGTGGGTGAAGC	AAGTGGTTTTGACCCCAATCATTAGAAGA	68°C	3	0.51	802	886	750-830
22	CEB326	6313702	33965	AAAGCAAGATGCATCTGGAACAAAG	TAAAGATCCTTGATGATTTTCTGAGGGATG	68°C	3	0.25	1408	802	1250-1680
22	CEB327	33983		CAGGAGCGGTGGGACTACACTT	GGCGGCTTCTCCACTCTCACT	68°C	1	0	793	793	800
22	CEB223	6313703	34031	AATACAGCACGATCGAATCTATCAGGACA	CCCTGTGGAGACAGTGTGTTGTGATG	68°C	2	0.5	1836	1864	1850-1950







**Figure 3** Criteria 1, 2, and 3 applied to the training set. For criteria 1 and 2, heterozygosity (28 individuals) versus HistoryR (criterion 1) or percentage of GC (%GC; criterion 2) are plotted. Correlations are significant at the 0.01 level. For criterion 3, HistoryR versus %GC is plotted, with different symbols representing the polymorphism. Lines represent the selected thresholds, and shaded areas contain the minisatellites selected by the criteria (criterion 1, HistoryR  $\geq 0.54$ ; criterion 2, %GC  $\geq 48\%$ ; criterion 3, criteria 1 and 2 combined). Plots show that criteria select most of the polymorphic minisatellites and eliminate a majority of monomorphs or slightly polymorphic ones.

We typed the eight most polymorphic minisatellites (i.e., with heterozygosity  $\geq 0.85$ ) in the eight largest CEPH families (102 children) to search for mutant alleles. Comparing the results obtained by PCR and Southern blotting shows that even when some larger alleles are missing in the PCR products, the estimated heterozygosity rate (see Methods) is close to the heterozygosity rate obtained with Southern blots. This helps validate the simplified PCR-based polymorphism measurement. Among the eight minisatellites (CEB202, CEB205, CEB250, CEB310, CEB269, CEB291, CEB305, CEB324), two showed mutant alleles (CEB205 and CEB310; Fig. 2B). Both yielded two mutant alleles among 204 meioses, that is, 102 children (mutation rate, 0.12% to 3.5%; 95% confidence interval). For minisatellite CEB205, one mutation event occurred in the mother and the other in the father, whereas for CEB310, both mutations occurred in the father. The remaining six minisatellites yielded no mutant allele among 102 children (mutation rate, 0 to 1.79%; 95% confidence interval). They were not investigated further but can not be strictly excluded from being hypermutable. The two minisatellites that appeared hypermutable among 102 children were then typed in more families (32 other reference CEPH families). For CEB205, one new mutant allele was found among 352 meioses (mutation rate, 0.54%; 95% confidence interval, 0.11% to 1.57%), but no other mutant allele was detected for CEB310 among 476 additional meioses (mutation rate, 0.29%; 95% confidence interval, 0.04% to 1.06%). Based on these results, CEB205 appears to be hypermutable. It is a GC-rich minisatellite with a unit length of 33 bp repeated 10 to 70 times, located at 1.5 Mb from the end of the chromosome 22 sequence. It seems to be part of a predicted coding region (gene *LOC129238*; see <http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=129238>, 31 July 2002 update).

## DISCUSSION

This study, performed on the scale of entire human chromosomes, provides a first global evaluation of minisatellite polymorphism based on genome sequence data. The repeats studied here, chosen by using a detailed definition that is more stringent than the broad definition mentioned in the Introduction, are, in majority (75%), polymorphic in the population investigated, and 42% have a heterozygosity value  $\geq 0.5$ . Minisatellites from chromosomes 21 and 22 are similar in physical distribution (higher frequency toward chromosome ends), sequence features, and polymorphism. Assuming that chromosomes 21 and 22 are representative of all human chromosomes and given that the two chromosomes represent  $\sim 2\%$  of the genome, we speculate that the entire human genome contains  $\sim 6,000$  minisatellites that match our definition, including 4,800 polymorphic and 2,500 very polymorphic ones. A few 10s of these might be expected to qualify as hypermutable loci. Be-

cause our definition precluded many other potentially polymorphic minisatellites, future research should seek to expand the category of minisatellites that are tested against our polymorphism prediction criteria.

## Predicting Polymorphism

We showed that using the sequence properties %GC and HistoryR effectively improves polymorphic minisatellite selection. With them, we reduce the number of minisatellites for typing by about half while increasing the frequency of repeats with heterozygosity  $\geq 0.5$  from the background rate of 43% to 59%. Internal conservation, used as a polymorphism predictor for microsatellites, is not applicable to minisatellites, presumably owing to the greater complexity of their mutation processes.

That %GC correlates with polymorphism is in agreement with earlier observations. Some of the first minisatellites to be characterized were detected via a shared 10- to 15-bp "core" sequence similar to the generalized recombination signal ( $\chi$ ) of *Escherichia coli* (GCTGTGG; Jeffreys et al. 1985). The majority of classical minisatellites (mostly polymorphic and/or hypermutable ones) are GC-rich, with a strong purine/pyrimidine strand asymmetry (Vergnaud and Denoeud 2000). In other genomes, though, (for instance bacterial genomes), %GC does not seem to be associated with minisatellites polymorphism (Le Fleche et al. 2001). Such a criterion may therefore not be universal, especially because GC content varies significantly across genomes.

The HistoryR criterion is based on the hypothesis that tandem repeats expand through multiple rounds of duplication, with the new copies sharing the mutations that occur before duplication, whereas unique mutations accumulate once the repeat is no longer evolving. For example (Fig. 1),

A		Test set	criterion1 +	criterion1 -	criterion2 +	criterion2 -	criterion3 +	criterion3 -
Number of repeats		67	38	29	57	10	32	35
% of the whole set		100%	57%	43%	85%	15%	48%	52%
monomorphs (het. = 0)		17	5	12	11	6	2	15
slightly polymorphic (0 < het. < 0.5)		21	12	9	19	2	11	10
moderately polymorphic (0.5 ≤ het. < 0.85)		24	16	8	23	1	15	9
highly polymorphic (het. ≥ 0.85)		5	5	0	4	1	4	1

B		Whole set	criterion3 +	criterion3 -	criterion4 +	criterion4 -
Number of repeats		118	61	57	41	75
% of the whole set		100%	52%	48%	35%	65%
monomorphs (het. = 0)		30	7	23	5	25
slightly polymorphic (0 < het. < 0.5)		39	20	19	11	27
moderately polymorphic (0.5 ≤ het. < 0.85)		41	28	13	21	19
highly polymorphic (het. ≥ 0.85)		8	6	2	4	4

**Figure 4** (A) Application of criteria 1 (HistoryR  $\geq 0.54$ ), 2 (%GC  $\geq 48\%$ ), and 3 (HistoryR  $\geq 0.54$  and %GC  $\geq 48\%$ ) to the test set. For each criterion, the distributions of minisatellites (from monomorphs to highly polymorphic) between positive (retained by the criterion) and negative (excluded by the criterion) sets are compared. All differences between sets + and - are statistically significant at the 0.01 level. (B) On the whole set, comparison of the results obtained with criterion 4 and criterion 3.

minisatellite CEB252 shows several redundant patterns of mutation, resulting in a high HistoryR score, whereas CEB233 shows no clear organization of mutations, resulting in a low HistoryR score.

This polymorphism criterion is likely to be applicable to any genome, even though the history reconstruction algorithm makes simplifying assumptions about the possible biological mechanisms involved in array expansion. These mechanisms, which include mutational events during mitotic replication and meiotic recombination, comprising both intraallelic and interallelic events, might occur independently or jointly. At present, there are no rules to predict which mechanism will occur preferentially at which locus (Maleki et al. 2002). Moreover, the individual mechanisms themselves are still poorly understood and, thus, impossible to model. Meiotic events, for instance, have been shown to result from the activity of nearby meiosis-specific double-strand break hot-spots. The nature of these sites, better known in yeast, is still unknown in the human genome (Debrauwere et al. 1999; Tamaki et al. 1999; Vergnaud and Denoeud 2000). In view of the current state of knowledge, it may be premature to hope for a perfect polymorphism predictor based on apparent array expansion.

### Use of Two Human Sequences to Select for Polymorphic Loci Is Problematic

The availability of two versions of the human genome sequence provides an additional avenue to improve polymorphic minisatellite identification. However, in the repeats stud-

ied here, selection based on reported length differences discarded half the highly polymorphic minisatellites and, in particular, the hypermutable one from chromosome 22. In both chromosomes, the number of loci with different predicted lengths in the HGP and Celera sequences that were nonetheless both found was significantly underrepresented. This is apparently owing to the lack of independence resulting from sharing of data during assembly of the Celera sequence. In addition, in both chromosomes, the number of loci in which only one or no predicted allele was found is overrepresented, apparently owing to assembly errors. Because the Celera sequence—which when not in agreement with the HGP data—usually provides

copy numbers unobserved in any allele, it appears that the Celera sequence, at least with respect to minisatellites, is more prone to assembly error. As a result of the lack of independence/assembly errors in the Celera/HGP data, polymorphism prediction based on sequence comparison did not perform as well as anticipated.

### One New Hypermutable Locus in a Coding Region

This study revealed one hypermutable minisatellite, CEB205, showing three mutant alleles among 278 children (mutation rate, 0.54%; 95% confidence interval, 0.11% to 1.57%). Interestingly, CEB205, with a 33-bp pattern, may be part of a coding region. The corresponding putative protein is 614 amino acids long, half of which are derived from the tandem repeat (11 codon repetition) at the N terminus. Of the minisatellites studied here, 26 among 60 (43%) on chromosome 21, and 22 among 67 (33%) on chromosome 22 belong to genes (i.e., exons, introns, or UTRs), as determined by sequence similarity analyses in the human genome sequence (using BLAST and <http://www.ncbi.nlm.nih.gov/genome/seq/>, release of November 2001). None except CEB205 appear to contribute to the coding sequence itself. Although the proportion of tandem repeats that contribute to coding regions is important in bacterial genomes, it is relatively low in the human genome, and CEB205 might represent the first known, coding hypermutable minisatellite.

CEB310—which exhibited meiotic mutation events, but which we do not here classify as hypermutable—is unusual in that its sequence is 80% AT. It is reminiscent of the tandem

**Table 2.** Success of the Public Human Genome Project (HGP) and Celera Sequences in Predicting Alleles That Were Actually Found to Occur

116 tandem repeats (predicted by both sequences)					
Predicted lengths match: n = 75		Predicted lengths differ: n = 41			
Allele found with predicted length:		Allele found with predicted length:			
Yes	No	Yes, both predictions	HGP prediction only	Celera prediction only	Neither prediction
70	5	10	26	0	5

repeats studied in Giraudeau et al. (1999), that is, minisatellites made up of degenerated microsatellite-like repeated units (in this case, [AC]m[AT]n). Although most hypermutable minisatellites known to date are GC-rich, some have been described as having a very high AT content, for instance, the one constituting the chromosomal fragile site FRA16B (Yu et al. 1997; Yamauchi et al. 2000). The highly polymorphic minisatellite MSY1, from human chromosome Y, is also very AT-rich (75% to 80%; Jobling et al. 1998).

Future research will expand the systematic exploration of human tandem repeat polymorphism by testing the %GC, HistoryR, and HGP/Celera criteria on other human chromosomes as the sequences are progressively finished and released (Deloukas et al. 2001).

## METHODS

### Constructing the Tandem Repeats Database

Tandem repeats were identified from chromosome 21 (Hattori et al. 2000) and chromosome 22 (Dunham et al. 1999) sequences by using the TRF software (Benson 1999) with the following options: alignment parameters of (2,3,5), minimum alignment score to report repeat of 50, maximum period size of 500. When the program reported redundant (overlapping) repeats, the redundancy was eliminated in the following way. For each group of overlapping repeats, two values were determined: Lmax, the maximal total length among the redundant alignments, and Mmax, the maximal percent matches among the redundant alignments with total length  $\geq 80\%$  of Lmax. Then, of all the alignments in the group with total length  $\geq 80\%$  of Lmax and percentage of matches  $\geq Mmax - 0.1$ , the one with smallest unit length was stored in the database. The nominal length of the stored repeat is the total length of the overlapping region, that is, from the first position of the first overlapping repeat to the last position of the last overlapping repeat. Twenty-two tandem repeats showed differences of  $>5\%$  between the nominal length and the length of the stored repeat, (the difference exceeded 10% in 14 cases, and 30% in three cases: CEB311, 33%; CEB320, 46%; and CEB327, 50%). For these latter three, TRF cut the repeats into two parts, which were combined for further analysis. Variation between nominal and stored size of repeats does not affect allele size prediction, which is based on length of sequence between primers. The database, publicly available at <http://minisatellites.u-psud.fr>, can be queried according to a number of simple features (e.g., total length, unit length, copy number, %GC) and provides links to repeat alignments and flanking sequence data as described previously (Le Fleche et al. 2001).

### PCR Typing of Minisatellites

DNA was provided by Centre d'Etudes du Polymorphisme Humain (CEPH; <http://www.cephb.fr/>). PCRs were performed in 15  $\mu$ L reactions, using 50 ng of genomic DNA, Roche long template PCR buffer (1.75 mM  $MgCl_2$ , 50 mM Tris-HCl at pH 9.2 and 25°C, 16 mM  $[NH_4]_2[SO_4]$ ), 0.033 U/ $\mu$ L Taq polymerase (Roche), 0.003 U/ $\mu$ L Pwo (Roche), 200  $\mu$ M of each dNTP (Amersham-Pharmacia biotech), and 0.6  $\mu$ M of each flanking primer (Table 1; primers were selected within the flanking sequences provided by TRF using Primer3 software: [http://www-genome.wi.mit.edu/cgi-bin/primer/primer3\\_www.cgi](http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi)). PCRs were cycled for 5 min at 96°C, then for 15 sec at 96°C: for 20 sec at annealing temperature (Table 1; this temperature was optimized for each primer pair by using the temperature gradient provided by MJResearch PTC200), for 5 min at 68°C for 30 cycles, and for 10 min at 68°C, on Perkin Elmer 9600 thermocycler or MJResearch PTC200. Samples were run through a 13-cm-long 1% standard agarose (Qbiogen) gel in  $0.5 \times$  TBE buffer at 10 V/cm for 1.5 h and visualized by

ethidium bromide staining using UV ( $1 \times$  TBE buffer is 89 mM Tris, 89 mM boric acid, 2 mM EDTA at pH 8).

### Polymorphism Measures

A population of 96 CEPH individuals (from the 40 reference families) were typed for minisatellite polymorphism. This population includes 13 mother/father/child trios and altogether comprises 76 unrelated individuals. The 76 unrelated individuals form subpopulation 1. A subset of 28 unrelated individuals forms subpopulation 2. The exact list of the 96 individuals typed is provided in Supplementary Table 2.

In this study, we examined only length polymorphism, not internal sequence variation. Two values, calculated on unrelated individuals, were used to quantify polymorphism: the number of alleles observed and the heterozygosity, calculated as  $1 - \sum f^2$ , where  $f$  are the allelic frequencies observed in the population of unrelated individuals. Heterozygosity represents the probability of having two different alleles. The simple PCR and ethidium bromide staining assay used here will usually detect only the smallest allele in individuals showing large length differences between alleles (as is often the case for highly polymorphic loci). The shorter allele often masks the longer one because it is easier to amplify. Such PCR artifacts are indicated with an asterisk in Table 1. They were detected because of the mother/father/child segregation controls and also because they do not satisfy the Hardy-Weinberg equilibrium, as tested with the HWE program, from the publicly available Linkage Utilities package (Ott 1999). For these loci, the heterozygosity value calculated from allelic frequencies was obtained by counting only one allele for individuals showing a single band (i.e., by assuming that the individual is heterozygous with one allele masked) instead of counting the same allele twice, as was done for loci in which homozygosity was not in question. The resulting heterozygosity value could be underestimated (if too many alleles are not seen), but it is sufficient to roughly evaluate the polymorphism.

### Mutation Rate Estimation

Mutation rate of the most polymorphic (i.e., potentially hypermutable) minisatellites was evaluated by a combination of Southern blot hybridization and PCR typings, in recognition of the "masking" phenomenon described above. Typings were performed by using DNA from the eight largest CEPH families (F102, F884, F1331, F1332, F1347, F1362, F1413, F1416). Five  $\mu$ g of DNA were digested with *AluI* (CEB202, CEB250, CEB269, CEB291) or *HinfI* (CEB205, CEB324, CEB305; Boehringer Mannheim), electrophoresed through a 1% agarose gel and transferred to nylon membranes (Nytran+, Schleicher and Schuell) under vacuum (Pharmacia Biotech). Probes were obtained from PCR products and recovered from agarose using QIAquick gel extraction kit (Qiagen). Probes were labeled with  $\alpha$ - $^{32}P$ dCTP (Amersham Pharmacia Biotech) by the random priming procedure (Feinberg and Vogelstein 1984). Hybridization was conducted as described in Vergnaud (1989) in an hybridization oven at 65°C. After hybridization, the filters were washed in  $1 \times$  SSC/0.1% SDS or  $0.1 \times$  SSC/0.1% SDS at 65°C. Membranes were revealed by using a phosphorimager (Storm 860 Molecular Dynamics).

### Sequence Characteristics of Repeats

The following sequence characteristics (calculated from the HGP sequence) were tested for correlation with either allele number or heterozygosity. Characteristics did not differ markedly when evaluated in the Celera sequence (in which differences with HGP typically involved deletion of adjacent copies reported in the HGP sequence):

1. Unit length: the length of the repetitive unit (consensus pattern).
2. Copy number: the number of copies of the repetitive unit.



3. Total length: the length of the entire tandem array.
4. Percent matches: the frequency at which a nucleotide at a position in one unit matches the corresponding nucleotide in the next unit (reading from left to right).
5. %GC: the percentage of nucleotides that are either G or C.
6. GC bias: strand asymmetry for G and C,  $|\%G - \%C|/(\%G + \%C)$ .
7. Purine/Pyrimidine bias: strand asymmetry for purines and pyrimidines,  $|\%Pur - \%Pyr|/(\%Pur + \%Pyr)$ .
8. Average entropy: from the columns of a multiple alignment of the repeat copies, the average, over all columns, of the entropy calculated from nucleotide frequencies.
9. HistoryR: described below.

HistoryR is derived from the tandem repeats history reconstruction algorithm (Benson and Dong 1999), a greedy algorithm that chooses a series of least-cost contractions to convert a multicopy tandem array into a single putative ancestral copy. Greedy algorithms are not guaranteed to find the overall least-cost solution, but testing has shown this approach to work very well on simulated sequences. Input is a multiple alignment,  $\mathbf{M}$ , of the individual copies in the repeat, with  $n$  rows (number of copies) and  $k$  columns (length of alignment).  $\mathbf{M}_{i,j}$  represents the  $i$ th row and  $j$ th column of  $\mathbf{M}$ , and each  $\mathbf{M}_{i,j}$  contains one of the alphabet symbols (A,C,G,T,-). In a contraction, two or more consecutive, equal-length subsequences (the contraction copies) are replaced by a single subsequence (the merged copy) of the same length (all subsequences selected have length equal to a multiple of  $k$ ). Each contraction reduces the number of rows in  $\mathbf{M}$ . If the contraction copies are identical, then one becomes the merged copy. Otherwise at every position at which the contraction copies differ, the merged copy contains the character that occurs most often, with ties being represented by an ambiguous character, that is, a set of all the most frequently occurring characters at that position. An ambiguous character created in one contraction may be converted to a single character in a subsequent contraction. This method is analogous to that used by Sankoff (1975; Sankoff and Rousseau 1975). The cost of a contraction is a ratio. The numerator is the cost of obtaining the contraction copies from the merged copy; that is, at each position of the merged copy, subtract the number of times the most frequent character occurs in the contraction copies from the total number of contraction copies, then sum all these differences. The denominator is the combined length of rows by which  $\mathbf{M}$  is reduced, that is, the length of all contraction copies minus the length of the merged copy.

History reconstruction yields four numerical values: (1) Max, the maximum possible history cost; (2) Min, the minimum possible history cost; (3) BinaryActual, the calculated history cost when the number of contraction copies in every contraction is restricted to exactly two; and (4) ManyActual, the calculated cost when the number of contraction copies is unrestricted. Max and Min are sums of column values from the original alignment  $\mathbf{M}$ . In the case of Max, the value of a single column is the number of characters that are not the most frequent character. Max is therefore the cost if the most frequent character is ancestral and if every character different from the ancestral character was produced by its own mutation. For Min, the value is one less than the number of distinct characters in a column, that is, at most four. Min is the history cost if every distinct character different from the ancestral character arose by a single mutation (with identical characters produced by duplication).

Combinations of the four numerical values were tested for polymorphism prediction in the training set and HistoryR, which produced the highest correlation with heterozygosity, was used for the remainder of the study. It is defined as

$$\text{History } R = \begin{cases} (Max - \text{BestActual}) / (Max - Min) & \text{when } Max \neq Min \\ 1 & \text{otherwise} \end{cases}$$

where BestActual is the minimum of BinaryActual and ManyActual. Usually, this was BinaryActual. The HistoryR value can be thought of as the proportion of mutations that could be accounted for by duplication that actually are. When  $Max \neq Min$ ,  $\text{History } R \leq 1$ , with a higher ratio indicating more mutations accounted for by duplications (Fig. 1). When  $Max = Min$ , each mutation is unique, and we arbitrarily set the ratio to one. This occurred in only one repeat with a total of four mutations. The history reconstruction program is freely available for interactive use at <http://tandem.biomath.mssm.edu/cgi-bin/history/history.exe>.

## Statistical Analysis

All statistical analysis was done with the SPSS program except for  $\chi^2$  tests which were done with StatXact 4. Correlations were determined by three methods: Pearson correlation, and nonparametric Kendall's  $\tau_b$  and Spearman's  $\rho$ . Correlations are considered significant at the 0.01 level (two-tailed) of the test statistics. Group comparisons were determined by first conducting two tests of normality, Kolmogorov-Smirnov and Shapiro-Wilkinson, on the values within each group. Values were assumed to be normally distributed unless the test statistic fell within the 0.05 level of significance. If the values were normally distributed in the two groups, then a  $t$  test was used to compare the means, which were judged significantly different at the 0.01 level of the statistic (two-tailed). If the values were not normally distributed in either of the two groups, then a nonparametric Mann-Whitney test was used to compare the distributions, which were judged significantly different at the 0.01 level of the statistic (two-tailed).

$\chi^2$  tests were used to analyze HGP/Celera prediction data for chromosomes 21 and 22 separately. The data were divided into three categories: (1) identical predictions/allele size detected, (2) different predictions/both alleles sizes detected, and (3) one or neither predicted allele size detected. Two estimates for frequency of unobserved alleles were used (in order to calculate the probability of alleles being detected): 10% which corresponds to the largest frequency in the population for which the chance of not appearing in our sample of 28 individuals is  $\geq 0.05$ , and an arbitrary low estimate of 1%. The probability of identical predictions in the HGP and Celera sequences was obtained by summing the estimated heterozygosity values calculated separately for each locus based on observed frequencies in our sample (equivalent to using the average observed heterozygosity over all loci).

## ACKNOWLEDGMENTS

We would like to thank Carol Bodian for extensive discussions and help with design of the  $\chi^2$  analysis. G.B. is supported in part by NSF grants CCR-0073081 and DBI-0090789. F.D. and G.V. are supported by grants from Délégation Générale de l'Armement.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

## REFERENCES

- Amarger, V., Gauguier, D., Yerle, M., Apiou, F., Pinton, P., Giraudeau, F., Monfouilloux, S., Lathrop, M., Dutrillaux, B., Buard, J., et al. 1998. Analysis of the human, pig, and rat genomes supports a universal telomeric origin of minisatellite sequences. *Genomics* **52**: 62–71.
- Appelgren, H., Cederberg, H., and Rannug, U. 1997. Mutations at the human minisatellite MS32 integrated in yeast occur with high frequency in meiosis and involve complex recombination events. *Mol. Gen. Genet.* **256**: 7–17.
- . 1999. Meiotic interallelic conversion at the human minisatellite MS32 in yeast triggers recombination in several chromatids. *Gene* **239**: 29–38.
- Baudat, F., Manova, K., Yuen, J.P., Jasin M., and Keeney, S. 2000. Chromosome synapsis defects and sexually dimorphic meiotic

- progression in mice lacking Spo11. *Mol. Cell* **6**: 989–998.
- Bell, G.I., Serby M.J., and Rutter, W.J. 1982. The highly polymorphic region near the human insulin gene is composed of simple tandemly repeating sequences. *Nature* **295**: 31–35.
- Benson, G. 1999. Tandem repeats finder: A program to analyze DNA sequences. *Nucleic Acids Res.* **27**: 573–580.
- Benson, G. and Dong, L. 1999. Reconstructing the duplication history of a tandem repeat. *Proc. Int. Conf. Intell. Syst. Mol. Biol.* **44**: 44–53.
- Bergerat, A., de Massy, B., Gadelle, D., Varoutas, P.C., Nicolas, A., and Forterre, P. 1997. An atypical topoisomerase II from archaea with implication for meiotic recombination. *Nature* **386**: 414–417.
- Buard, J. and Vergnaud, G. 1994. Complex recombination events at the hypermutable minisatellite CEB1 (D2S90). *EMBO J.* **13**: 3203–3210.
- Buard, J., Bourdet, A., Yardley, J., Dubrova Y., and Jeffreys, A.J. 1998. Influences of array size and homogeneity on minisatellite mutation. *EMBO J.* **17**: 3495–3502.
- Debrauwère, H., Buard, J., Tessier, J., Aubert, D., Vergnaud, G., and Nicolas, A. 1999. Meiotic instability of human minisatellite CEB1 in yeast requires DNA double-strand breaks. *Nat. Genet.* **23**: 367–371.
- Deloukas, P., Matthews, L.H., Ashurst, J., Burton, J., Gilbert, J.G., Jones, M., Stavrides, G., Almeida, J.P., Babbage, A.K., Bagguley, C.L., et al. 2001. The DNA sequence and comparative analysis of human chromosome 20. *Nature* **414**: 865–871.
- Dubrova, Y.E. and Plumb, M.A. 2002. Ionising radiation and mutation induction at mouse minisatellite loci: The story of the two generations. *Mutat. Res.* **499**: 143–150.
- Dubrova, Y.E., Jeffreys, A.J., and Malashenko, A.M. 1993. Mouse minisatellite mutations induced by ionizing radiation. *Nat. Genet.* **5**: 92–94.
- Dubrova, Y.E., Nesterov, V.N., Krouchinsky, N.G., Ostapenko, V.A., Vergnaud, G., Giraudeau, F., Buard J., and Jeffreys, A.J. 1997. Further evidence for elevated human minisatellite mutation rate in Belarus eight years after the Chernobyl accident. *Mut. Res.* **381**: 267–278.
- Dunham, I., Shimizu, N., Roe, B.A., Chissole, S., Hunt, A.R., Collins, J.E., Bruskewich, R., Beare, D.M., Clamp, M., Smink, L.J., et al. 1999. The DNA sequence of human chromosome 22. *Nature* **402**: 489–495.
- Feinberg, A.P. and Vogelstein, B. 1984. Addendum: a technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **137**: 266–267.
- Fondon III, J.W., Mele, G.M., Brezinschek, R.I., Cummings, D., Pande, A., Wren, J., O'Brien, K.M., Kupfer, K.C., Wei, M.H., Lerman, M., et al. 1998. Computerized polymorphic marker identification: Experimental validation and a predicted human polymorphism catalog. *Proc. Natl. Acad. Sci.* **95**: 7514–7519.
- Giraudeau, F., Petit, E., Avet-Loiseau, H., Hauck, Y., Vergnaud, G., and Amarger, V. 1999. Finding new human minisatellite sequences in the vicinity of long CA-rich sequences. *Genome Res.* **9**: 647–653.
- Giraudeau, F., Taine, L., Biancalana, V., Delobel, B., Journel, H., Moncla, A., Bonneau, D., Lacombe, D., Moraine, C., Croquette, M.F., et al. 2001. Use of a set of highly polymorphic minisatellite probes for the identification of cryptic 1p36.3 deletions in a large collection of patients with idiopathic mental retardation: Three new cases. *J. Med. Genet.* **38**: 121–125.
- Hattori, M., Fujiyama, A., Taylor, T.D., Watanabe, H., Yada, T., Park, H.S., Toyoda, A., Ishii, K., Totoki, Y., Choi, D.K., et al. 2000. The DNA sequence of human chromosome 21: The chromosome 21 mapping and sequencing consortium. *Nature* **405**: 311–319.
- Heale, S.M. and Petes, T.D. 1995. The stabilization of repetitive tracts of DNA by variant repeats requires a functional mismatch repair system. *Cell* **83**: 539–545.
- Jeffreys, A.J., Wilson, V., and Thein, S.L. 1985. Hypervariable “minisatellite” regions in human DNA. *Nature* **314**: 67–73.
- Jeffreys, A.J., Tamaki, K., MacLeod, A., Monckton, D.G., Neil, D.L., and Armour, J.A.L. 1994. Complex gene conversion events in germline mutation at human minisatellites. *Nat. Genet.* **6**: 136–145.
- Jobling, M.A., Bouzekri, N., and Taylor, P.G. 1998. Hypervariable digital DNA codes for human paternal lineages: MVR-PCR at the Y-specific minisatellite, MSY1 (DYF155S1). *Hum. Mol. Genet.* **7**: 643–653.
- Keeney, S., Giroux, C.N., and Kleckner, N. 1997. Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* **88**: 375–384.
- Le Fleche, P., Hauck, Y., Onteniente, L., Prieur, A., Denoeud, F., Ramisse, V., Sylvestre, P., Benson, G., Ramisse, F., and Vergnaud, G. 2001. A tandem repeats database for bacterial genomes: application to the genotyping of *Yersinia pestis* and *Bacillus anthracis*. *BMC Microbiol.* **1**: 2.
- Maleki, S., Cederberg, H., and Rannug, U. 2002. The human minisatellites MS1, MS32, MS205 and CEB1 integrated into the yeast genome exhibit different degrees of mitotic instability but are all stabilised by RAD27. *Curr. Genet.* **41**: 333–341.
- May, C.A., Jeffreys, A.J., and Armour, J.A.L. 1996. Mutation rate heterogeneity and the generation of allele diversity at the human minisatellite MS205 (D16S309). *Hum. Mol. Genet.* **5**: 1823–1833.
- Murray, J., Buard, J., Neil, D.L., Yeramian, E., Tamaki, K., Hollies, C.R., and Jeffreys, A.J. 1999. Comparative sequence analysis of human minisatellites showing meiotic repeat instability. *Genome Res.* **9**: 130–136.
- Nakamura, Y., Leppert, M., O'Connell, P., Wolff, T., Holm, T., Culver, M., Martin, C., Fujimoto, E., Hoff, M., Krumlin, E., et al. 1987. Variable number of tandem repeat (VNTR) markers for human gene mapping. *Science* **235**: 1616–1622.
- NIH/CEPH collaborative mapping group. 1992. A comprehensive genetic linkage map of the human genome. *Science* **258**: 67–83.
- Ott, J. 1999. *Analysis of human genetic linkage*, 3d ed. Johns Hopkins University Press, Baltimore, MD.
- Romanienko, P.J. and Camerini-Otero, R.D. 2000. The mouse Spo11 gene is required for meiotic chromosome synapsis. *Mol. Cell* **6**: 975–987.
- Sankoff, D. 1975. Minimal mutation trees of sequences. *J. Appl. Math.* **28**: 35–42.
- Sankoff, D. and Rousseau, P. 1975. Locating the vertices of a Steiner tree in an arbitrary metric space. *Math. Programming* **9**: 240–246.
- Strand, M., Prolla, T.A., Liskay, R.M., and Petes, T.D. 1993. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* **365**: 274–276.
- Tamaki, K., May, C.A., Dubrova, Y.E., and Jeffreys, A.J. 1999. Extremely complex repeat shuffling during germline mutation at human minisatellite B6.7. *Hum. Mol. Genet.* **8**: 879–888.
- Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., et al. 2001. The sequence of the human genome. *Science* **291**: 1304–1351.
- Vergnaud, G. 1989. Polymers of random short oligonucleotides detect polymorphic loci in the human genome. *Nucleic Acids Res.* **17**: 7623–7630.
- Vergnaud, G. and Denoeud, F. 2000. Minisatellites: Mutability and genome architecture. *Genome Res.* **10**: 899–907.
- Vergnaud, G., Mariat, D., Apiou, F., Aurias, A., Lathrop, M., and Lauthier, V. 1991. The use of synthetic tandem repeats to isolate new VNTR loci: Cloning of a human hypermutable sequence. *Genomics* **11**: 135–144.
- Weber, J.L. 1990. Informativeness of human (dC-dA)n (dG-dT)n polymorphisms. *Genomics* **7**: 524–530.
- Wren, J.D., Forgacs, E., Fondon III, J.W., Pertsemilidis, A., Cheng, S.Y., Gallardo, T., Williams, R.S., Shohet, R.V., Minna, J.D., and Garner, H.R. 2000. Repeat polymorphisms within gene regions: phenotypic and evolutionary implications. *Am. J. Hum. Genet.* **67**: 345–356.
- Yamauchi, M., Tsuji, S., Mita, K., Saito, T., and Morimyo, M. 2000. A novel minisatellite repeat expansion identified at FRA16B in a Japanese carrier. *Genes Genet. Syst.* **75**: 149–154.
- Yu, S., Mangelsdorf, M., Hewett, D., Hobson, L., Baker, E., Eyre, H.J., Lapsys, N., Le Paslier, D., Doggett, N.A., Sutherland, G.R., et al. 1997. Human chromosomal fragile site FRA16B is an amplified AT-rich minisatellite repeat. *Cell* **88**: 367–374.

## WEB SITE REFERENCES

- <http://minisatellites.u-psud.fr>; the tandem repeats database.
- <http://tandem.biomath.mssm.edu/cgi-bin/history/history.exe>; history reconstruction program
- <http://www.ncbi.nlm.nih.gov/genome/seq/>; Human Genome Sequencing at NCBI.
- [http://www-genome.wi.mit.edu/cgi-bin/primer/primer3\\_www.cgi](http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi); Primer3 primer picking software.
- <http://www.cephb.fr>; Centre d'Etudes du Polymorphisme Humain.
- <http://www.ncbi.nlm.nih.gov/LocusLink/LocRpt.cgi?l=129238>; locuslink at NCBI, predicted gene LOC129238.
- <http://www.ncbi.nlm.nih.gov/SNP/index.html>; dbSNP home page.

Received July 1, 2002; accepted in revised form January 28, 2003.